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Missouri State Public Health Laboratory

Instructions for the collection and submission of specimens for avian influenza testing to the Missouri Department of Health Laboratory.

The Missouri Department of Health State Public Health Laboratory (MSPHL) provides laboratory testing services for persons with suspected infection with highly pathogenic avian influenza (H5N1). Consultation with the MSHPL at 573-751-0633 is required prior to collecting clinical specimens. Please refer to the infection control precautions for H5N1 (CDC interim recommendations published on February 3, 2004) at http://www.cdc.gov/flu/avian/professional/han020302.htm.

It is recommended that multiple specimens be collected, and that multiple specimen types be considered. If confirmation testing is needed, it is preferable to use fresh clinical material.

Please refer to the MSPHL web page at http://www.dhss.missouri.gov/Lab/Virology/RespiratoryVirusTesting.html for current laboratory information.

I. RESPIRATORY SPECIMENS

Nasopharyngeal wash/aspirates are the specimen of choice for detection of most respiratory viruses and are the preferred specimen type for children aged <2 years. Respiratory specimens for detection of most respiratory pathogens, and influenza in particular, are optimally collected within the first 3 days of the onset of illness.

A. Collecting specimens from the upper respiratory tract

1. Nasopharyngeal wash/aspirate

- Have the patient sit with head tilted slightly backward.
- Instill 1 ml–1.5 ml of nonbacteriostatic saline (pH 7.0) into one nostril. Flush a plastic catheter or tubing with 2 ml–3 ml of saline. Insert the tubing into the nostril parallel to the palate. Aspirate nasopharyngeal secretions. Repeat this procedure for the other nostril.
- Collect the specimens in sterile vials. Label each specimen container with the patient's ID number and the date collected.
- Refrigerate samples after collecting. Ship using cold packs to keep the sample at 4°C.

2. Nasopharyngeal or oropharyngeal swabs

- Use only sterile dacron or rayon swabs with plastic shafts. Do **not** use calcium alginate swabs or swabs with wooden sticks, as they may contain substances that inactivate some viruses and inhibit PCR testing.
- To obtain a **nasopharyngeal swab**, insert a swab into the nostril parallel to the palate. Leave the swab in place for a few seconds to absorb secretions. Swab both nostrils.
- To obtain an **oropharyngeal swab**, swab the posterior pharynx and tonsillar areas, avoiding the tongue.
- Place the swabs immediately into sterile vials containing 2 ml of viral transport media. Break the

applicator sticks off near the tip to permit tightening of the cap. Label each specimen container with the patient's name, ID number and the date the sample was collected.

- Refrigerate samples after collecting. Ship using cold packs to keep the sample at 4°C..

(All types of respiratory specimens may be used in RT-PCR tests. Fresh-frozen unfixed tissue specimens may also be submitted for RT-PCR).

B. Collecting specimens from the lower respiratory tract

1. Broncheoalveolar lavage, tracheal aspirate, or pleural fluid tap

- During bronchoalveolar lavage or tracheal aspirate, use a double-tube system to maximum shielding from oropharyngeal secretions.
- Centrifuge half of the specimen, and fix the cell pellet in formalin. Place the remaining unspun fluid in sterile vials with external caps and internal O-ring seals. If there is no internal O-ring seal, then seal tightly with the available cap and secure with Parafilm®. Label each specimen container with the patient's ID number and the date the sample was collected.
- Refrigerate samples after collecting. Ship using cold packs to keep the sample at 4°C.

2. Sputum

- Educate the patient about the difference between sputum and oral secretions.
- Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile screw-cap sputum collection cup or sterile dry container.
- Refrigerate samples after collecting. Ship using cold packs to keep the sample at 4°C.

II. BLOOD COMPONENTS

Both acute and convalescent serum specimens should be collected for antibody testing. Collect convalescent serum specimens 2–4 weeks after the onset of illness. To collect serum for antibody testing:

- Collect 5 ml–10 ml of whole blood in a serum separator tube. Allow the blood to clot, centrifuge briefly, and collect all resulting sera in vials with external caps and internal O-ring seals. If there is no internal O-ring seal, then seal tightly with the available cap and secure with Parafilm®.
- The minimum amount of serum preferred for each test is 200 microliters, which can easily be obtained from 5 ml of whole blood. A minimum of 1 cc of whole blood is needed for testing of pediatric patients. If possible, collect 1 cc in an EDTA tube and in a clotting tube. If only 1cc can be obtained, use a clotting tube.
- Label each specimen container with the patient's ID number and the date the specimen was collected.
- Refrigerate and ship with cold packs to keep the sample at 4°C. If frozen, ship on dry ice. Do not freeze whole blood.

III. AUTOPSY SPECIMENS

CDC can perform immunohistochemical (IHC) staining for influenza A (H5) viruses on autopsy specimens. Viral antigens may be focal and sparsely distributed in patients with influenza, and are most frequently detected in respiratory epithelium of large airways. Larger airways (particularly primary and segmental bronchi) have the highest yield for detection of influenza viruses by IHC staining. Collection of the appropriate tissues ensures the best chance of detecting the virus by (IHC) stains.

- If influenza is suspected, a minimum total of 8 blocks or fixed-tissue specimens representing samples from each of the following sites should be obtained and submitted for evaluation:
- Central (hilar) lung with segmental bronchi
- Right and left primary bronchi
- Trachea (proximal and distal)
- Representative pulmonary parenchyma from right and left lung

In addition, representative tissues from major organs should be submitted for evaluation. In particular, for patients with suspected myocarditis or encephalitis, specimens should include myocardium (right and left ventricle) and CNS (cerebral cortex, basal ganglia, pons, medulla, and cerebellum). Specimens should be included from any other organ showing significant gross or microscopic pathology.

Specimens may be submitted as:

- Fixed, unprocessed tissue in 10% neutral buffered formalin, or
- Tissue blocks containing formalin-fixed, paraffin-embedded specimens, or
- Unstained sections cut at 3 microns placed on charged glass slides (10 slides per specimen)
- Specimens should be sent at room temperature (**NOT FROZEN**).
- Fresh-frozen unfixed tissue specimens may be submitted for RT-PCR.
- Include a copy of the autopsy report (preliminary, or final if available), and a cover letter outlining a brief clinical history and the submitter's full name, title, complete mailing address, phone, and fax numbers, in the event that CDC pathologists require further information. Referring pathologists may direct specific questions to CDC pathologists. The contact number for the Infectious Disease Pathology Activity is 404-639-3133, or the pathologists can be contacted 24 hours a day, 7 days a week through the CDC Emergency Response Hotline at 770-488-7100.

IV. SHIPPING INSTRUCTIONS

The Missouri State Public Health Laboratory (MSPHL) will supply mailers suitable for the collection and transportation of avian influenza specimens. These are similar to the kits that have been distributed for rash investigation and SARS testing. It is specifically recommended that the virus isolation kits supplied for seasonal influenza surveillance not be used.

Specimens should be stored under refrigeration (4^0C) and shipped on cold packs (not wet ice). Samples (such as fresh-frozen autopsy samples for RT-PCR or other clinical materials) may be frozen at –70º if the package is to be held prior to shipment.

All specimens should be submitted to the MSPHL, including any that are being submitted to the CDC. This will ensure proper documentation and specimen packaging procedures are maintained.

If mailers from the SPHL are not used, specimens should be packaged and shipped in a container suitable for the transportation of etiologic agents (infectious substances). Protocols for standard interstate shipment of etiologic agents should be followed, and are available at http://www.cdc.gov/od/ohs/biosfty/shipregs.htm. All shipments must comply with current DOT/IATA shipping regulations.

The MSHPL provides courier services for the transportation of specimens to the laboratory. If the state courier is not used, specimens should be sent by priority shipping for receipt within 24 hours. The MSHPL shipping address is:

Missouri State Public Health Laboratory
Attention: Virology Unit
307 West McCarty
Jefferson City, MO 65101